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EVALUATING DISTINCTNESS & EVOLUTIONARY SIGNIFICANCE OF PREBLE'S MEADOW JUMPING MOUSE :

PHYLOGEOGRAPHY OF MITOCHONDRIAL DNA NON-CODING REGION VARIATION

FINAL REPORT

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Evaluating Distinctness & Evolutionary Significance of Preble's Meadow Jumping Mouse:

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SUMMARY

We have generated molecular genetic data for a total of 92 jumping mice including 71 sampled from 20 populations in Colorado and four populations in Wyoming, and 21 specimens representing three reference groups of closely related taxa. DNA sequences obtained for a 433 basepair (bp)-long portion of the mitochondrial DNA non-coding region (sometimes referred to as the D-loop) in all samples indicate that a group of populations ranging from southeastern Albany County, Wyoming, south along the Front Range of the Rocky Mountains to western Las Animas County, Colorado, form a coherent group, both genetically and geographically. This group, which we refer to as the "Preble's group" is distinct from four of five other populations sampled by live trapping or from museum specimens and may be somewhat differentiated from the fifth of these. The Preble's group is also clearly distinct from two of the three reference groups and less markedly differentiated from the third.

Phylogenetic analyses based on these data are still at a preliminary stage and indicate that the Preble's group of populations is most closely allied with, and not strongly differentiated from, samples of *Z. h. intermedius* from Minnesota. Of the four populations that appear to be distinct from the Preble's group, two sampled near the Colorado-New Mexico border are closely allied with museum specimens identified as *Z. h. luteus* from New Mexico. Two others from the vicinity of Cheyenne, Wyoming, are most similar to representative samples of *Z. princeps* from western Larimer Co., Colorado and are next most closely allied with a *Z. princeps* specimen from northern New Mexico. A suggestion from our analysis is that *Z. hudsonius* from Indiana (possibly either *Z. h. americanus* or *Z. h. intermedius* based on distribution) may be the most ancestral of the populations and taxa sampled and may have shared a common ancestor with progenitors of forms presently known as *Z. princeps princeps* and *Z. h. luteus*.

BACKGROUND

Recent applications of molecular genetic methods in systematics, population genetics, and conservation biology have demonstrated that variation in DNA markers can be highly informative in the evaluation of genetic distinctness and evolutionary significance, two important determinants in decisions regarding application of the Federal Endangered Species Act of 1973 as amended and interpreted by the U. S. Congress. The study described here sought to discover whether and how molecular data might support and help objectify the view, based on other more traditional criteria, of the Preble's mouse as an evolutionary unit distinct from other species and subspecies in the genus *Zapus*.

Work funded by the Colorado Division of Wildlife (hereafter CDW) and performed by Biosphere Genetics, Inc. has evaluated the ability of three different approaches to quantifying variation in the DNA molecule to provide reliable and informative data addressing issues of key importance in the decision of whether or not to list the Preble's mouse. Previous reports to CDW have summarized our finding that RAPD (randomly amplified polymorphic DNA) markers are not sufficiently repeatable for use in the context of this study. Methods to assay variation in another class of genetic markers found in nuclear DNA called microsatellites or simple sequence repeats, although potentially promising, are not yet sufficiently well developed and validated for zapodids to apply at this time. A third approach, DNA sequencing of the mitochondrial DNA non-coding region which includes the D-loop, emerged from preliminary work as the method most appropriate and informative, in combination with other more traditional criteria, for the determination of whether populations of the Preble's meadow jumping mouse (*Zapus hudsonius preblei*) constitute one or more distinct evolutionary units with significance warranting protection under the Federal Endangered Species Act. Results based on sequence variation in the mitochondrial DNA non-coding region assayed in a subsample of the 54 mice presumed to be Preble's obtained during the summer of 1996 indicated: (1) that 7 populations sampled at sites ranging from Longmont to Colorado Springs in Colorado varied relatively little from one another, (2) that two other populations sampled in Las Animas County near Lake Dorothey were similar to one another but differed quite markedly from the first group, and (3) that none of the individuals sampled in Laramie County in Wyoming could be assayed using the techniques successfully applied to the other samples, suggesting that this population may also differ to some degree from those in Colorado.

Field surveys of the occurrence of the Preble's meadow jumping mouse conducted by several individuals and organizations during the summer of 1996 were extended to additional sites in the summer of 1997. CDW sponsored some of these efforts and coordinated the collection, recording and delivery of tissue samples for DNA analyses performed by Biosphere Genetics, Inc., also under contract with the Division. In addition, the U. S. Air Force supported DNA analysis of samples obtained from trapping efforts during the summer of 1996 on the Warren Air Force Base and the inclusion of reference material representing two additional subspecies of *Zapus hudsonius*, *Z. h. palustris*, and *Z. h. campestris*.

METHODS

Tissue Sampling and Storage

A sampling protocol and data forms for using in obtaining samples for DNA analysis were developed for CDW and distributed to all parties participating in the coordinated trapping effort. The disseminated protocol, adapted and updated from the U.S. Fish and Wildlife Service standardized protocol, described handling and instrument cleansing procedures to minimize contamination of the sample with human or other mammalian DNA. Very small samples of tissue were obtained from live-trapped

specimens of *Zapus* by using a tagging punch (National Band and Tag Co., Newport, KY) to remove one or more plugs of tissue from the outer portion of each mouse's ear. In some cases, ear tissue was obtained by clipping or notching the ear with scissors. Tissue plugs or pieces from a given animal were placed in a cryogenic screw-cap vial filled 1/3-1/2 full with 95% ethanol and labeled with an alpha-numeric code intended to uniquely specify trapping location and the individual animal caught. Hair samples were collected by some participants and placed either in a separate labeled vial or in the same vial containing the ear tissue. Samples were conveyed to CDW and accumulated into lots for delivery to BGI. When samples were transported from the field or shipped by common carrier, they were at ambient temperature. Upon receipt at BGI, samples were kept refrigerated at 4°C until the time of DNA extraction. Specimens obtained from field surveys and miscellaneous museum specimens used to expand coverage of the potential range of the taxa examined in this study are listed in Table 1 of the Appendix to this report.

Reference specimen tissues used for comparison with field-sampled jumping mice were provided from four separate museum collections (see Appendix I, Table 2) as toes clipped from museum specimens or as small pieces of internal organs maintained in the museum's frozen collection, usually at -70°C. Toe clips were shipped at ambient temperature and refrigerated at 4°C upon receipt. Frozen specimens were shipped on dry ice and extracted upon receipt with any remaining tissue being transferred to ethanol for further storage. Reference specimens are listed in Table 2 of the Appendix to this report.

DNA Extraction & Purification

We extracted DNA from plugs or pieces of ear tissue using the Hair Lysis Buffer (HLB) method similar to that of Greer et al. (1995). The tissue was rinsed, either with a jet of triple-distilled water or by agitation in a drop of distilled water on Parafilm®, then placed in a volume of HLB and incubated at 56° C with periodic agitation for 10-12 hours. In most cases, this process completely digested the sample leaving only a trace of particulate matter suspended or settled in the bottom of the tube. With the particulates centrifuged to the bottom, aliquots of the extract were withdrawn for fractionation and for purification prior to use in DNA amplifications. Raw DNA extracts were run out on 1.5% agarose gels and stained with ethidium bromide to quantify the DNA by comparison with staining intensity of Lambda Hind III fragments run on the same gel. We used Prep-A-Gene™ (Bio-Rad, Hercules, CA) to purify the DNA preparations before sequencing.

Amplification of Mitochondrial DNA Non-coding Region Sequences

We began our examination of the mitochondrial non-coding region using the primers designated as ECO-THR (L15996) and BAM-TDKD (H16401) to amplify an approximately 550 bp-long segment between the threonine tRNA gene near the cytochrome b end of the non-coding region and the TDKD site approximately half way through the non-coding region.[1] This primer combination, first used to study human

[1] The ECO- and BAM- portions of these primer designations refer to the addition of restriction enzyme sequences at the 5' end of the primer sequence. These "tails" are not essential for the present application and we will use primer designations with and without this notation interchangeably in this report.

populations (Vigilant et al. 1989) had worked well in studies of other mammals and typically revealed amounts of sequence variation appropriate to differentiating subspecies and geographically isolated populations. Because the non-coding region includes a structure described as a displacement or "D" loop known from work on human and cattle DNA, we will occasionally refer to the region of DNA studied here as the D-loop.

Amplifications with the above primers were performed in 12.5 µl reaction volumes using 1 µl of template DNA, 1 unit of the DNA polymerase, Klentaq-1 (Ab Peptides, St. Louis, MO), and other reaction components in standard proportions. A Techne PHC-2 thermocylcer was used to implement a program using an initial denaturation at 94°C for 3 minutes followed by 42 cycles with denaturing at 94° for 45 seconds., annealing at 55° for 1 minutes, and extension at 72° for 1 minutes followed by a final extension at 72° for 5 minutes. Products were fractionated on 1.5% agarose gels. Amplification of the 550 bp D-loop fragment was successful, but primer dimer and secondary products also appeared, sometimes in considerable amounts. We experimented with a variety of modifications to obtain cleaner product (primer reduction, additions of $MgCl_2$ at various levels, alternative forms of DNA polymerase, etc) and were ultimately able to obtain product with a minimum of these additional elements in many, but not all, samples of mice presumed to be *Z. h. preblei*.

Following the optimization effort, revised reaction conditions were used to assess D-loop fragment amplification success with all new DNA extracts in 12.5 µl reactions, then applied with slight modifications to the thermocyler program to amplify targeted products in 50 µl reaction volumes to obtain sufficient quantities for automated sequencing. Two or three 50 µl reactions were run for each sample, depending on prior estimates of the amount of template available in individual DNA extractions. The quantity and quality of product generated by each reaction was assessed by agarose gel fractionation. Reaction volumes were combined when necessary, then cleaned with the solid-phase reversible immobilization method (DeAngelis et al. 1995) using carboxyl coated magnetic particles (PerSeptive BioSystems Inc, Cambridge, MA). Cleaned, resuspended DNA products were then dried down to a pellet in the bottom of a 1.5 ml eppie tube for shipping by overnight service at ambient temperature to an automated sequencing service.

Resolution of Amplification Problems

Some DNA extracts, most notably those of *Z. princeps* and of putative *Z. h. preblei* populations sampled at the Warren Air Force Base in Wyoming, did not amplify well or at all with the ECO-THR/BAM-TDKD primer combination. We first suspected that this occurred because the TDKD primer designed from sequence information on the human D-loop was not finding sufficient complementarity on the heterologous primer site in at least some *Zapus* populations or taxa. Conventional wisdom suggested that the ECO-THR primer site should be highly conserved in vertebrates. Based on this reasoning, we designed two new primers for the middle of the D-loop. We examined D-loop sequences available from Genbank for rat (*Rattus norwegicus*), mouse (*Mus musculus*), vole (*Clethrionomys glareolus)*, gopher (*Thomomys bottae*), and kangaroo rat (*Dipodomys ordii)* as well as our own sequence data for *Zapus*. Sequences for the

relevant portion of the D-loop for *Mus*, *Clethrionomys* and *Zapus* were used to identify a primer site most likely to be reasonably conserved in muroid and dipodoid rodents. The primers designed for this new site are designated PRDL L-15738 (forward primer) and PRDL H-15720 (reverse primer). We had these primers synthesized (Operon Technologies, Alameda, CA) for testing and possible use in resolving the difficulties mentioned above. The relative positions and priming directions of all primers are shown in Figure 1.

We used PRDL H-15720 in combination with ECO-THR in an attempt to amplify those samples that had not amplified with the ECO-THR/BAM-TDKD primer combination. We also attempted to amplify the entire D-loop by using ECO-THR in combination with PHE-rev H29, a primer previously designed for general use in amplifying portions of the D-loop in mammals (A. Gavazzi, unpublished). Finally we tested PRDL L-15738 in combination with PHE-rev H29 for the purpose of generating D-loop fragments spanning the TDKD site for automated sequencing of this site in *Zapus*. Testing of these primers on *Zapus* DNA extracts revealed that: 1) the ECO-THR/PRDL H-15720 primer combination amplified in only two of 20 samples and did nothing to solve the previous difficulties encountered with the ECO-THR/BAM-TDKD combination; 2) the ECO-THR/PHE-rev H29 combination produced a fragment ca. 1500 bp long, apparently spanning the D-loop successfully in some cases, but not with the DNA extracts that had been previously problematic, and 3) the PRDL L-15738/PHE-rev H29 combination produced multiple bands in some cases and no amplification in others. We inferred from these results that our original problem in amplifying some *Zapus* samples, most notably those assigned to *Z. princeps* and the samples from the Warren Air Force Base, was not with the TDKD site, but rather with annealing of the ECO-THR primer designed for a conserved section of the human threonine tRNA gene to the heterologous sequence in *Zapus*.

Reexamination of the sequences successfully obtained in preliminary work on *Zapus* suggested that a primer site that would be more highly conserved between *Zapus* and other mammals might be present internal to the one primed by ECO-THR. Further testing showed that an available primer designated PROC, for priming of a sequence on the proline tRNA gene, when used in combination with the BAM-TDKD primer, was uniformly very successful in amplifying a ca. 433 bp fragment (excluding primer sequences) of the D-loop. While we have been reluctant to give up on efforts to access information about variation existing in and around the THR site, the PROC/BAM-TDKD primer combination was clearly the tool of choice for compiling the dataset required for this study within the time frame required. The results presented in this report are for the 433 bp segment of the D-loop falling between the proximal ends of these primer sites.

Automated Sequencing

DNA sequencing of both the 550 bp and 433 bp fragments of the D-loop generated as described above was performed by The University of Maine DNA Sequencing Core Facility using an ABI model 373A Stretch DNA sequencer. Results of one sequencing pass in each direction were received by e-mail as attached files in ABI format. Pherograms in these files were viewed with the program Chromas (v. 1.4, Conor

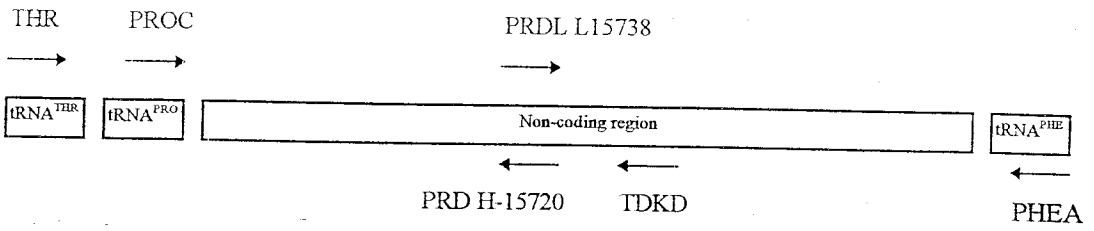

Fig. 1. Relative positions of the primers used in this study for amplification of the mitochondrial DNA non-coding region, which includes the D-loop. Primers THR and TDKD were from Vigilant et al. (1995), PHE-rev H29 was from Anita Gavazzi and primers prdl L-15738 and prdl H-15720 are two new primers designed for this study to work with Zapus and other rodents. Primers are used in pair-wise combinations to amplify segments of DNA lying between their positions on this simplified map. The relative positions of coding and non-coding regions are indicated by labeled areas.

McCarthey, Griffith University, Brisbane, Queensland, Australia) and machine-assigned sequences were exported as text files for use in subsequent analysis.

Data Analysis

Text files containing the two sequencing reads generated from opposite ends of the amplified fragment were entered into the program MacDNASIS (v. 1, Hitachi Software Engineering Co., Ltd., San Bruno, CA) and combined to obtain a consensus sequence for the amplified region of each individual mouse sampled. Sequences obtained from both THR-TDKD and PROC-TDKD amplifications were used as available. However, since the more inclusive THR-TDKD sequences were not available for individuals assigned to *Z. princeps* and samples ultimately found to be more closely related to *Z. princeps* than to the Preble's samples, and since the longer sequences were of variable quality at the beginning of the 5' to 3' read in a number of the Preble's samples, we used only the 433 bp sequence available in all samples in all subsequent data analysis.

Consensus sequences were aligned manually using the multiple sequence option of MacDNASIS. The program MacClade (v. 3, Maddison, 1992) was used to create a data matrix of character states for 58 variable sites in the aligned sequences for phylogenetic analyses. Phylogenetic trees were generated using the parsimony algorithm in PAUP (v. 3.1.1, Swofford, 1993). To expedite review of various options for the rooting of trees, one individual was selected as most representative of each population (fewest changes from the most commonly shared state at the 58 variable sites treated as characters in the DNA sequence) and used in the depiction of most-parsimonious trees and in bootstrap analyses. Bootstrap analyses used the 50% majority rule with 100 replications.

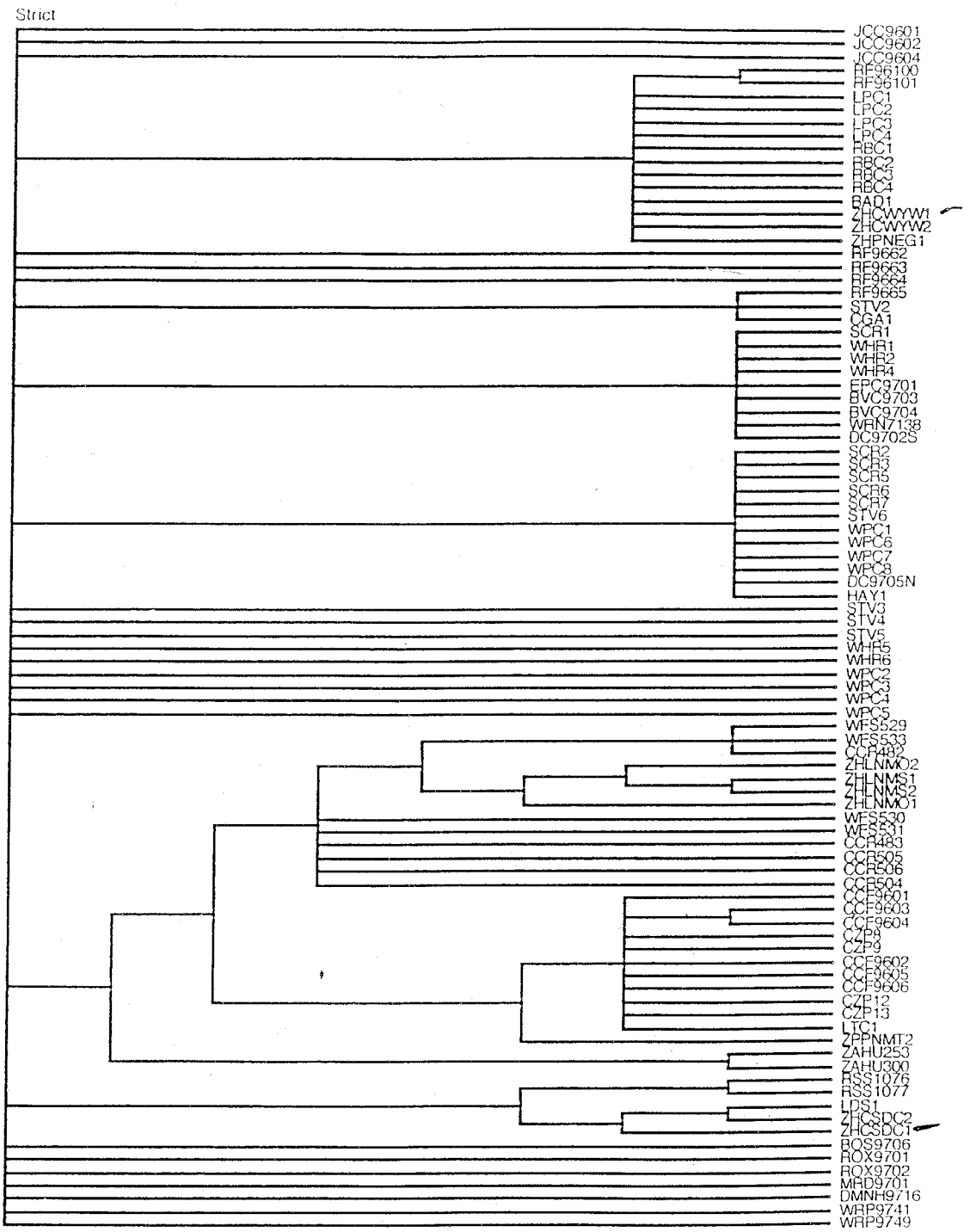

Figure 2. Strict consensus tree, identical to the single most parsimonious tree generated using the "branch and bound" option of PAUP v. 3.1.1 (Swofford 1993) on sequences for all samples analyzed. Length of this shortest tree is 61 evolutionary steps; consistency index (CI) = 0.869. Although not supported by bootstrap analysis in the context of phylogenetic analysis, sub-groupings of populations considered here to be part of the Preble's group suggest that population subdivision may be discernable by more appropriate population-analytic methods.

RESULTS

Figure 2 illustrates phylogenetic relationships among all samples included in the study to date, inferred from maximum parsimony analysis (length = 61 steps, consistency index [CI] = 0.869). Bootstrap analysis for the entire dataset was not practical for this report. However, bootstrap analyses were performed for various outgroup scenarios using one individual from each population as described above. No outgroup was designated for the analysis shown in Figure 2.

The important relationships in Figure 2 are perhaps more easily seen in the unrooted, circular format tree generated using one representative individual from each population (Figure 3). No meaning is attached to the length of branches in this tree. The 19 branches that join the baseline in the center directly (11 o'clock to 5 o'clock on the diagram) and four more samples that are shown grouped together and joining the baseline with a bootstrap value of 53 (indicating little support for treating this group of samples as distinct from the other 19) form a relatively homogenous group. Also not strongly differentiated is the group of three representing reference samples of *Z. h. intermedius* from Minnesota and *Z. h. campestris* from South Dakota as well as an individual museum specimen designated as only *Z. hudsonius* from Johnson County, Wyoming. Bootstrap values indicate strong support for recognizing samples of *Z. princeps*, *Z. h. luteus*, and populations affiliated with them in the diagram as distinct from the Preble's group. Further observations following from the analysis are discussed in the following section.

DISCUSSION

Preliminary data analyses conducted before all new populations sampled during the summer of 1997 and reference material had been analyzed examined three options for the rooting of the phylogenetic tree. When the Indiana population of *Z. hudsonius* was assigned as the outgroup (one most parsimonious tree, length = 63, CI = 0.921), the strong relationships in the tree were the same ones seen in the tree generated when no outgroup was assigned (Figures 2 and 3). Essentially, populations of jumping mice ranging from southeastern Albany County in Wyoming south along the Front Range of the Rocky Mountains to western Las Animas County in Colorado were seen to form a coherent group within which separate populations or groups of populations were not strongly differentiated. Designating *Z. princeps* as the outgroup resulted in Indiana *Z. hudsonius* appearing as the sister group to the assemblage of presumed Preble's populations with the populations from the Colorado-New Mexico border and the Warren Air Force Base joining the tree at an intermediate level. Bootstrap values for the resulting tree were uniformly high, but we have not yet resolved doubts about whether *Z. princeps* can properly be treated as ancestral to *Z. hudsonius*. Finally, designating *Z. hudsonius intermedius* from Minnesota as the outgroup in earlier analyses resulted in a tree in which all the branches to the Preble's populations samples joined the baseline as undifferentiated from the outgroup.

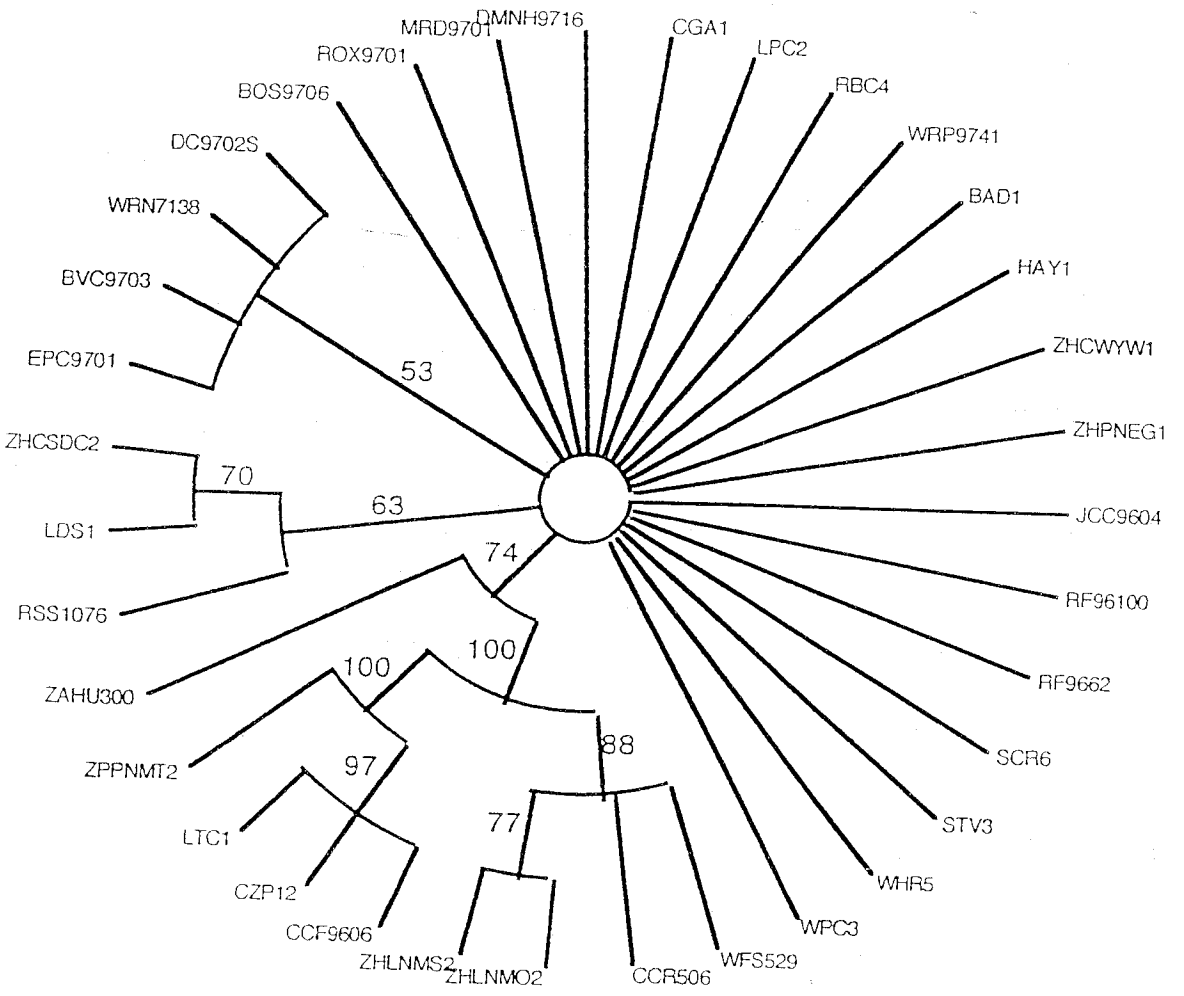

Figure 3. Bootstrap consensus tree (50% majority rule, 100 replications), based on analysis of single most-representative individual from each population or reference group, obtained with no outgroup designated. Bootstrap values are shown next to relevant branches in the tree. Bootstrap values greater than 90% indicate those relationships which are strongly supported.

There are several notable observations for individual population samples among our findings based on analyses completed in late November, 1997. We summarize these here in list form with population/sample codes in parentheses referring to designations in the tables of the Appendix:

1. The six jumping mice caught at the Warren Air Force Base (CCF) in the summer of 1996 (identified by field workers as Preble's) and an individual jumping mouse caught in Weld County, Colorado just south of Cheyenne, Wyoming (LTC), are indistinguishable from reference samples of *Z. princeps* (CZP) provided by Dr. Bruce Wunder from Larimer County, Colorado. Bootstrap values indicate that this group is, in turn, most closely affiliated with, but distinct from, a sample of *Z. princeps* from Taos County, New Mexico.

2. The eastern-most sample identified in the field as *Z. h. preblei*, a single individual from Elbert County, Colorado (HAY), is confirmed to belong in the Preble's group by DNA sequence obtained from a sample of three hairs.

3. Jumping mice caught at two sites in the Lake Dorothey area on the Colorado-New Mexico border (CCR and WFS), suspected on the basis of morphological traits to be similar *to Z. h. luteus* (Cheryl A. Jones, personal communication), are confirmed to be so on the basis of the non-coding region sequence data. There is also a reasonably strong indication (bootstrap value = 88) that these two populations and the *Z. h. luteus* samples are, together, likely to have shared a common ancestor with progenitors of *Z. princeps* reference samples and the populations sampled north (CCF) and south (LTC) of Cheyenne, Wyoming. This finding is in contrast to the conclusions of Hafner et al. (1981) (but see next comment).

4. A suggestion from our analysis (not strongly supported by bootstrap analysis) is that *Z. hudsonius* from Indiana (possibly either *Z. h. americanus* or *Z. h. intermedius* based on distribution) may be the most ancestral of the populations and taxa sampled and may have shared a common ancestor with progenitors of forms presently known as *Z. princeps princeps* and *Z. h. luteus*. We speculate that the forms recognized as subspecies of *Z. princeps* and *Z. hudsonius* present today in the Rocky Mountain and Northern Great Plains region may be the derivatives of two separate range expansions occurring at different times during the Pleistocene.

5. Two reference samples obtained from the Denver Museum of Natural History, originally identified as *Z. princeps* from the San Isabel National Forest in western Las Animas County, appear on the basis of non-coding region sequence data to belong to the Preble's group.

6. A single jumping mouse specimen obtained by Chris Garber from the Medicine Bow National Forest in Wyoming, identified as a Preble's mouse by Dr. David Armstrong on the basis of his analysis of poorly preserved skeletal material, is confirmed to be a Preble's by DNA sequencing from a piece of preserved skin.

7. One reference collection sample identified as *Z. h. pallidus* from Garden County, Nebraska (ZHPNEG), and two samples identified as *Z. h. campestris* from Weston

County, Wyoming (ZHPWYW), are indistinguishable from other samples in the Preble's group in the present analysis.

8. Two reference collection samples identified as *Z. h. campestris* from Custer County, South Dakota, are found to be most similar to a museum specimen identified only to species level as *Z. hudsonius* from the vicinity of Buffalo in Johnson County, Wyoming.

Further Research Needs

Additional sample materials and analyses may be required to achieve a rigorous view of the relationships of the group referred to here as Preble's to other subspecies and species of the genus *Zapus* in North America. The choice of an appropriate outgroup remains unresolved, and could benefit from further input from those knowledgeable about evolution and morphology of the Zapodids in North America and elsewhere. Based on extensive work on limb myology of the Dipodoidea (Stein, 1990), the superfamily to which the genus and the rest of the family Zapodidae belong, the genus *Sicista* (the birch mice), represented by species in Europe and Asia, would appear to be an appropriate outgroup. However, divergence between *Zapus* and *Sicista* may be too great to be effectively measured by variation in the D-loop. Another North American genus, *Napeozapus*, has been suggested as a possible outgroup for this analysis. However, slightly greater adaptation for saltatorial ability and a higher tooth count in *Napeozapus* than in *Zapus* would suggest that the former is derived rather than ancestral to the latter. Sequence information for the cytochrome b gene potentially forthcoming from other researchers (T. Yates, personal communication) may be helpful in sorting out relationships at the species and higher taxonomic levels and may be a useful complement to the non-coding region sequence data in elucidating relationships potentially traceable to Pleistocene glaciation events. We have begun to examine a portion of the cytochrome b gene and the neighboring threonine gene in a separate study.

The DNA sequence results reported here indicate the need for a reexamination of external and skeletal morphology in *Zapus*. In at least a few cases (DMNH, LDS), the sequence data suggest that even species level identifications cannot always be made unambiguously in the field. Patterns of morphological variation across the range of the species and subspecies of interest may be such that more careful examination of museum accessions is to be recommended as well. One promising dental character that may distinguish *Z. hudsonius* from *Z. princeps* is the presence of a deep anteromedian fold in the anteroconid of the molar m1 in the former as described by Klingener (1963) and applied in an analysis of cave floor faunal remains by Hafner (1993). It will be interesting to learn what this character may say about the taxonomic identity of the samples referenced above and about specimens available in museum collections for the areas near the northern and southern boundaries of the Preble's mouse range.

Further analysis of molecular markers at the species, subspecies, and population levels is needed to confirm the findings suggested by this study and to further delineate patterns of variation bearing on conservation and management of Z. h. preblei. We have material in hand to continue to expand sample sizes for a number of the populations already surveyed and our initial contacts with several museums in the context of the

present study indicate that sample sizes of comparative taxa can also be increased for at least some parts of the relevant subspecis ranges. Because there has been relatively little recent survey activity in Wyoming and only a few reference specimens from museum collections were included in this study, the northern extent of populations assignable to the Preble's group is poorly defined. New trapping survey initiatives and DNA analysis of more material available from museums would address this situation. We also recommend that a nuclear DNA dataset be generated to complement the mitochondrial DNA dataset. A portion of our work not reported here has evaluated two classes of DNA markers potentially appropriate to this task and begun applications of microsatellite methods to development of markers likely to be applicable both to phylogenetic analysis of relationships at the species and subspecies level and to population genetic analyses supporting assessment and monitoring of population differentiation and gene flow, genetic parameters of population viability, and mating system analysis.

We expect that further examination of the results obtained in this study, both by ourselves and others, will lead to a more comprehensive definition of research needs and priorities along two possibly divergent paths, one inclined to address fundamental issues of systematics and biology, and the other focused more sharply on the near-term requirements that may be imposed by the outcome of the pending decision on the status of the Preble's mouse by the U.S. Fish and Wildlife Service. We urge that planning attention be devoted to providing for both ongoing research and for integration of assessment and monitoring activities into any prospective recovery effort. Molecular ecology has much to offer in both areas of endeavor.

Conclusion

Based on results to date, we conclude that mitochondrial DNA non-coding region (D-loop) sequence data appear consistent with the view that a geographically contiguous set of populations previously recognized as the Preble's meadow jumping mouse (*Z. h. preblei*) form a homogenous group recognizably distinct from other nearby populations and from another geographically-adjacent species of the genus.

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Appendix

Tables in this appendix contain information on the populations and species samples included in this study. All samples received for which DNA was extracted are listed. Those for which DNA sequence data was successfully obtained for the analyses reported in the main document are indicated as explained in each table.

Additions and corrections were still being received as the final draft of this report was going to press. Questions about incomplete or inaccurate data should be referred to the Colorado Division of Wildlife, Terrestrial Section, Attn: Judy Sheppard.

Table 1. Sampling locations for jumping mice analyzed as putative Preble's mice as (Zapus hudsonius preblei). This table includes both samples obtained by live-trapping during the summers of 1996 and 1997, and samples from museum specimens not identified to subspecies or otherwise designated as reference material (included in Table 2). Numbers of samples for which DNA sequence data were successfully obtained in time for the analyses presented in the main body of the report are shown in parentheses under the number of samples processed. The individuals included in the analyses are indicated by an asterix in the column headed "Original sample #s."

Sample Code	Locality/site name & description or other source information	No.	Original sample #s	1/4-1/4 Section	Lat-Long coordinates	UTM Coords.	Collector(s)	Sponsoring/participating organization(s)
BAD	Badwater Creek, Natrona Co., Wyoming. [elev. and quadrangle N/A]	1 (1)	CU 13469*	N/A	N/A	N/A	P. Robinson & E. Anderson	Univ. of Colorado Museum, Boulder, CO
BOS	South Boulder Creek, near intersection with East Boulder Ditch, on City of Boulder Open Space. Boulder Co., CO. 5300 ft. Lousiville Quadrangle, T 1 S, R 70 W	13 (1)	BOS9702 BOS9703 BOS9704 BOS9705 BOS9706* BOS9707 BOS9708 BOS9709 BOS9710 BOS9711 BOS9712 BOS9715 BOS9716	3, W 1/2 3, W 1/2 3, W 1/2 3, W 1/2 3, W 1/2 3, W 1/2 3, W 1/2 3, W 1/2 3, W 1/2 3, W 1/2 3, W 1/2 3, W 1/2 3, W 1/2	39°59' 30" N 105°12'55" W	4818450.00, 4427090.00 4818450.00, 4427090.00 4818450.00, 4427090.00 4818450.00, 4427090.00 4818450.00, 4427090.00 4818450.00, 4427090.00 4818450.00, 4427090.00 4818450.00, 4427090.00 4818450.00, 4427090.00 4818450.00, 4427090.00 4818450.00, 4427090.00 4818450.00, 4427090.00 4818450.00, 4427090.00	C. Meaney & N. Clippinger	Colorado Division of Wildlife Carron Meaney, Consultant
BVC	Beaver Creek, 2 1/2 miles SW of Monument, El Paso Co., CO. 7000 ft. Palmer Lake quadrangle, T 11 S, R 68 W	4 (2)	BVC9701 BVC9702 BVC9703* BVC9704*	29,NE 1/4 29,NE 1/4 29,NE 1/4 29,NE 1/4	39°03'27.2" 104°53'49.8"	5088970.00, 4323168.00 5088970.00, 4323168.00 5088970.00, 4323168.00 5088970.00, 4323168.00	N. Clippinger	Colorado Division of Wildlife Carron Meaney, Consultant
CCF	Crow Creek, Warren Air Force Base, Laramie Co., WY. [elevation and quadrangle N/A] T 14 N, R 67 W	6 (6)	CCF9601* CCF9602* CCF9603* CCF9604* CCF9605* CCF9606*	27 27 27 27 27 27	N/A	N/A	C. Flemming C. Pague	Colorado Natural Heritage Program U. S. Air Force
CCR	Chicorica Creek, Lake Dorothey State Wildlife Area, Las Animas Co., CO [Elev.=?]	6 (4)	482* 483* 503 504 505* 506*	N/A	39°00'02" 104°21'39"	N/A	C. A. Jones	Denver Museum of Natural History
CGA	N Branch, Middle Fork, Lodgepole Creek, Pole Mt. Unit, Medicine Bow National Forest, ca. 2 mi. S, 15 mi. E Laramie, Albany Co., WY, 7600 ft. [Quadrangle = N/A]	1 (1)	[Specimen tag]	T 15 N R 71 W sec 11	N/A	N/A	C. Garber (collector) D. Armstrong	University Museum, Univ. of Colorado
DC	Deadman Creek, U. S. Air Force Academy, El Paso Co., CO, [elevation and quadrangle N/A]	5 (2)	97DC02N 97DC03N 97DC02S* 97DC05S* 97DC07S	N/A	N/A	4318360.00, 513700.00 4318360.00, 513700.00 4318360.00, 513700.00 4318380.00, 513650.00 4318300.00, 513650.00	P. Schuerman, S. Ask, J. Hobert	Colorado Natural Heritage Program

Table 1 continued (2nd page).

EPC	East Plum Creek, 6500 ft. Douglas Co., CO. Dawson Butte quadrangle, T 9 S, R 67 W	3 (1)	EPC9701* EPC9702 EPC9703	9, E 1/2 9, E 1/2 9, E 1/2	39°16'46.3" N 104°53'44.2" W	5090040.00, 4347802? 5090040.00, 4347802? 5090040.00, 4347802? 5090040.00, 4347802?	A Deans	Colorado Division of Wildlife Carron Meaney, Consultant
HAY	Hay Gulch, tributary to Running Creek near Parker, Elbert Co., CO. 6,225 ft. Cabin Gulch quadrangle, T 7 S, R 64 W.	1 (1)	HAY-1*	E 1/2	N/A	540660.00, 4368470.00 (?)	A. Diens	Colorado Division of Wildlife Carron Meaney, Consultant
JCC	Coal Creek, Ransom/Edwards Homestead Ranch, Jefferson Co., CO. [Elev. = ?] (Eldorado Springs, CO) T 2 S, R 70 W	4 (3)	9601* 9602* 9603 9604*	7,8,17,18	[Information needed from collectors]	[Information needed]	C. Flemming C. Pague	Colorado Natural Heritage Program
LDS	Lake De Smet near Buffalo, Johnson Co., WY. [elevation and quadrangle N/A]	1 (1)	CU 15132*	N/A	N/A	N/A	A. Crockett J.M. Merritt D. Armstrong	Univ. of Colorado Museum, Boulder, CO
LPC	Lone Pine Creek, Lower Cherokee State Wildlife Area, Larimer Co., CO. 6200 ft. Livermore Mountain quadrangle, T 10 N, R 71 W	10 (4)	LPC9701* LPC9702* LPC9703* LPC9704* LPC9705 LPC9706 LPC9707 LPC9708 LPC 9709 LPC 9710	4,SW 1/4 9 NW, NW 4, SW 1/4 9 NW, NW 4, SW 1/4 9 NW, NW 4, SW 1/4 9 NW, NW 4, SW 1/4 9 NW, NW	40°46'11.0" 105°21'36.7"	4696010.00, 45132560.00 4696010.00, 45132560.00 4696010.00, 45132560.00 4696010.00, 45132560.00 4696010.00, 45132560.00 4696010.00, 45132560.00 4696010.00, 45132560.00 4696010.00, 45132560.00 4696010.00, 45132560.00 4696010.00, 45132560.00	A. Deans	Colorado Division of Wildlife Carron Meaney, Consultant
LTC	Lone Tree Creek, at I-25, northern portion Weld Co., CO. 5,900 ft. Carr Southwest quadrangle, T. 11-12 N, R. 67 W.	1 (1)	LTC-1*	NE NE, SE SE	40°57' 5.8" N 104°55'29.3" W	5063300.00, 45333900.00	A. Deans	Colorado Division of Wildlife Carron Meaney, Consultant
MRD	Marshall Road, Dry Creek Ditch #2, between Marshall road and South Boulder Creek, Boulder Co., CO. 5700 ft. Louisville quadrangle, T 1 S, R 70 W	1 (1)	MRD9701*	16, NW 1/4	39°58'1.6" 105°13'59.7"	4798400.00, 44242200.00	A. Deans	Colorado Division of Wildlife Carron Meaney, Consultant
RBC	Rabbit Creek, Lower Cherokee SWA ca. 9 mi. NW Livermore, Larimer Co. CO. 6300 ft. Livermore Mountain quadrangle ,T 10 N, R 71 W	8 (4)	RBC9701* RBC9702* RBC9703* RBC9704* RBC9705 RBC9706 RBC9707 RBC9708	21, NW 1/4 18, SE 1/4 15, NW 1/4 10, SW 1/4 10, SW 1/4 10, SW 1/4 10, SW 1/4 10, SW 1/4	40°49'25.6" 105°21"8.6"	4715010.00, 45212170.00 4715010.00, 45212170.00 4715010.00, 45212170.00 4702850.00, 45194120.00 4715010.00, 45212170.00 4715010.00, 45212170.00 4702850.00, 45212170.00 4702850.00, 45212170.00	A. Deans	Colorado Division of Wildlife Carron Meaney, Consultant
RF	Rocky Flats Environmental Technology Site, Jefferson Co, CO. 5780 ft. Louisville, CO, T 2 S, R 70 W	10 (6)	ZAHU 96-62* ZAHU96-63* ZAHU96-64* ZAHU96-65* HU96-100* HU96-101* RF-97-102 RF-97-103 RF-97-104 RF-97-105	 11, 5 W 11, 5 W 11, 5 W 11, 5 W	N/A	 4832800.00, 44148500.00 4832800.00, 44148500.00 4832800.00, 44148500.00 4832800.00, 44148500.00	T. Ryon	PTI Environmental Services

Table 1, continued (3rd page).

ROX	Roxborough State Park near junction of Willow Creek and Little Willow Creek, Douglas Co., CO. 7300 ft. Kassler quadrangle, T 7 S, R 69 W	2 (2)	ROX9701* ROX9702*	24, E 1/2 24, E 1/2	39° 25'44.5" 105°03'48.9" 39° 26'10.1" 105°04'24.0"	4945290.00, 43643880.00 4936880.00, 43651170.00	A. Deans	Colorado Division of Wildlife Carron Meaney, Consultant
SCR	Smith Creek, U. S. Air Force Academy, El Paso Co., CO. 6,860 ft. Monument quadrangle, T 12 S, R 67 W	6 (6)	SCR1* SCR2* SCR3* SCR5* SCR6* SCR7*	6 NE, SE 1/4	N/A	N/A	C. Meaney	Colorado Division of Widlife
STV	St. Vrain R., Boulder County Open Space, Hygiene, Boulder Co., CO. 5,060 ft. Hygiene quadrangle, T 3 N, R 70 W	6 (5)	STV1 STV2* STV3* STV4* STV5* STV6*	36, SE 1/4	N/A	N/A	C. Meaney	Colorado Division of Wildlife
WFS	West Fork Schachheim Creek, Lake Dorothey State Wildlife Area, Las Animas Co., CO [Elev. = ?] [Quadrangle = ?], [Twnshp./Rng./Sec = ?]	4 (4)	530* 531* 532* 533*	N/A	37°00'25" 104°22'32"	N/A	C. A. Jones	Denver Museum of Natural History
WHR	Woodhouse Ranch, Douglas Co., CO. 5,760 ft. Kassler quandrangle, T 7 S, R 68 W	7 (5)	WHR1* WHR2* WHR3 WHR4* WHR5* WHR6* WHR7	36, NE 1/4, SW 1/4	N/A	N/A	C. Meaney and N. Clippinger	Colorado Division of Wildlife
WPC	West Plum Creek, Douglas Co., CO [Detailed description needed from collector] [Elev. = ?] [Quadrangle = ?], T 7 S, R 68 W	8 (8)	WPC1* WPC2* WPC3* WPC4* WPC5* WPC6* WPC7* WPC8*	36, SW 1/4, E 1/2	[Need info. from collector]	[Information needed]	?	?
WRN	Monument Creek near confluence with Pine Creek, N. side of Woodman Road, El Paso Co., CO.	5 (1)	97WRN130 97WRN138* 97WRN142 97WRN143 97WRN75	N/A	N/A	4309600.00, 5139400.00 4309580.00, 5139400.00 4309550.00, 5139400.00 4309550.00, 5139400.00	P. Schuerman S. Ask J. Hobert	
WRP	White Ranch Park, Ralston Creek, off Hwy 43 north of Golden, Jefferson Co., Colorado, 5740 ft. Ralston Buttes quadrangle, T 2 S, R 70 W	3 (2)	WRP9725 WRP9741* WRP9749*	31		04774210.00, 44084950.00 04774210.00, 44084950.00 04774210.00, 44084950.00	Jan Peterson	Colorado Natural Heritage Program

Table 2. Reference specimens of jumping mice provided by museums and other investigators, used as representatives of recognized species and subspecies of the genus *Zapus* for comparison with specimens listed in Table 1.

Sample Code	Specimen information provided by source individual or institution	No.	Original sample nos.	1/4-1/4 Section	Lat-Long	UTM Coordinates	Collector/Source	Cooperating Institution
CZP	Neota Creek, FR 156 off Colo Hwy 14, Larimer Co, CO. 10,000 ft. Assigned to *Zapus princeps*	4 (4)	CZP 8* CZP 9* CZP 12* CZP 13*	N/A	N/A	N/A	B. Wunder	Colorado State University
DMNH	Purgatoire Campground, 2625 m. San Isabel National Forest, Las Animas Co., Colorado. Assigned to *Zapus princeps* at DMNH	2 (1)	7916* 7917	N/A	37°15'00" N 105°6'30"W	N/A	C. A. Jones	Denver Museum of Natural History
RSS	*Zapus hudsonius* samples provided by Bruce Wunder, Anoka County, Minnesota. Blaine, NE 1/4 of NE, Sec. 1, T31N, R23W Presumed to be *Z. h. intermedius*	2 (2)	RSS 1076* RSS 1077*	N/A	N/A	N/A	B. Wunder Robert Sikes Elmer Burney	Colorado State University University of Minnesota
ZAHU	*Zapus hudsonius* samples provided by Bruce Wunder , origin in Indiana; may be Z. h. *intermedius* or *Z. h. americanus*	2 (2)	ZAHU-253* ZAHU-300*	N/A	N/A	N/A	B. Wunder collected by John Whittaker	Colorado State University
ZHCSDC	*Zapus hudsonius campestris* from Custer Co., SD, provided by the Museum of Natural History, Univ. of Kansas	2 (2)	109994* 109995*	N/A	N/A	N/A	R. Timm T. Holmes	Natural History Museum, Univ. of Kansas, Lawrence
ZHCWYW	*Zapus hudsonius campestris* from Weston Co., WY, provided by the Museum of Natural Hisotry, Univ. of Kansas	2 (2)	42469* 42470*	N/A	N/A	N/A	R. Timm T. Holmes	Natural History Museum, Univ. of Kansas, Lawrence
ZHLNMO	*Zapus hudsonius (luteus)* from Otero Co., NM	2 (2)	NK 878* NK 879*	N/A	N/A	N/A	T. Yates B. Gannon	Museum of Southwestern Biology, Univ. of New Mexico, Albuquerque, NM
ZHLNMS	*Zapus hudsonius (luteus)* from Sandoval Co., Fenton Lake, NM	2 (2)	NK 3835* NK 3837*	N/A	N/A	N/A	T. Yates B. Gannon	Museum of Southwestern Biology, Univ. of New Mexico, Albuquerque, NM
ZHPNEC	*Zapus hudsonius pallidus* from Cherry Co., NE	2 (0)	-87043 87044	N/A	N/A	N/A	R. Timm T. Holmes	Natural History Museum, Univ. of Kansas, Lawrence
ZHPNEG	*Zapus hudsonius pallidus* from Garden Co., NE	2 (1)	115762* 115763	N/A	N/A	N/A	R. Timm T. Holmes	Natural History Museum, Univ. of Kansas, Lawrence
ZPPCOG	*Zapus princeps princeps* from Ptarmigan Camp, Grand Co., CO	2 (0)	CU 14912 CU 14913	N/A	N/A	N/A	A. deQueiroz D. Armstrong	Univ. of Colorado Museum, Boulder, CO
ZPPNMT	*Zapus princeps princeps* from Taos Co., NM	2 (1)	NK 811 NK 814*	N/A	N/A	N/A	T. Yates B. Gannon	Museum of Southwestern Biology, Univ. of New Mexico, Albuquerque, NM

N/A = not available for this report. In some cases the indicated information may be obtained from the person or institution supplying the material analyzed or can be inferred from other available information.